THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 50, No. 5

Printed in U.S.A.

EFFECT OF PROLONGED ADMINISTRATION OF TESTOSTERONE ON THE EPIDERMIS OF FEMALE RATS*

HERSCHEL S. ZACKHEIM, M.D.

The present communication reports the effect of prolonged systemic administration of testosterone on the epidermis of female rats. While the stimulatory effect of testosterone on the sebaceous glands is well known, the effect of testosterone on the epidermis is less well defined.

PROCEDURE

Twelve female albino Sprague-Dawley rats, approximate weight 200 gms, age 8 weeks, were treated with aqueous testosterone suspension (Schering) 2.5 mg, 3×/week, intra-muscularly (IM) for 25 weeks. An equal number of female albino Sprague-Dawley rats of the same weight were similarly injected with an equal volume of distilled water (0.1 cc) without testosterone.

The effect of the testosterone was determined from biopsy specimens taken from the abdominal skin at 2 weeks and then at approximately 4 week intervals. The specimens were taken at corresponding areas from the abdomen from the testosterone and control rats. The specimens were approximately 5 x 10 mm in size and were fixed in 10% buffered formalin. Paraffin sections 7 μ thick were stained with hematoxylin and eosin, toluidine, blue, and Alcian Blue-Nuclear fast red.

Both the testosterone treated and control rats were also given weekly X-ray treatments of 500 r to the mid-back for the 25 week period. The radiation factors were: 30 kv, half value layer .052 mm Al, skin target distance 15 cm, field size 5 cm diameter through a cone. Since the 50% tissue depth dose of this quality radiation is approximately 0.6 mm (1), the abdominal skin may be regarded as unaffected by the X-ray. This was confirmed by the absence of changes in the abdominal skin of the X-irradiated control rats as compared to non-irradiated rats. The effect of testosterone on the incidence and course of X-ray induced skin changes and neoplasms in these rats will be reported in a separate communication. None of the irradiated rats had developed tumors at the end of the 25 week treatment period.

Epidermal thickness is given as the distance from the base of the basal layer to the surface of the stratum granulosum (Table I). Measurements were taken only from inter-follicular epidermis. Six measurements were made at approximately equal intervals along the length of each section and then averaged. The second line of values in Table I indicates the number of rats whose epidermis was 33% or more thicker than the average epidermal thickness of the controls at that time period. The average epidermal thickness for the testosterone treated and control rats and the standard deviation of these measurements is stated. The bottom line indicates the average percentage increase in thickness of the testosterone as compared to the control rats. Our values for epidermal thickness in untreated animals are somewhat less than those reported by Ebling (2) whose biopsy specimens were taken from the back. We took great care not to measure areas that were cut at a bias. At any rate, the same technique was used for both the testosterone and the control rats and it is the difference between the two that is the significant point of this study. The slides were labelled in such a way that the identity of the specimens was unknown at the time the measurements were made.

RESULTS

Epidermis. Although at 4 weeks in 2 of 6, and at 8 weeks in 2 of 12 rats the epidermis was 33% or more thicker than the average thickness of the controls, it was not until 12 weeks that there was a significant and persistent increase in epidermal thickness in the testosterone treated group. This continued for the

Accepted for publication November 16, 1967.
Supported by PHS Research Grant CA 10048 from the National Cancer Institute, National Institutes of Health, Bethesda, Maryland.
* From the Department of Dermatology, Stanford University School of Medicine, Palo Alto, California.

TABLE I

Effect of testosterone on epidermal thickness

(2.5 mg, 3×/week for 25 weeks)

	0 wk	2 wk	4 wk	8 wk	12 wk	16 wk	20 wk	25 wk
No. rats biop.	6	5	6	12	6	6	6	10
Epid inc. >33%	—	0	2	2	4	6	5	8
Test. epid μ	12.8	14.9	18.1	16.5	20.2	27.0	23.5	20.7
S.D.	±1.46	±2.3	±3.4	±2.7	±4.0	±3.5	±4.0	±3.4
Control epid μ	13.2	14.5	13.5	14.6	13.5	13.5	14.4	13.6
S.D.	±0.58	±1.4	±0.79	±1.0	±0.62	±0.44	±0.7	±0.9
% inc. thickness	—	2.8	34.1	13.0	50.0	100.0	63.2	52.2

remainder of the 25 week period but was true of all the rats only at 16 weeks at which time the average epidermal thickness of the testosterone rats also reached its maximum of 27.0 microns.

Histologically, the most striking feature of the hyperplasia was a marked accentuation of the granular cell layer (Figs. 1a and 1b). This was evident even when the differences in epidermal thickness were not strikingly apparent, and provided an early clue to the effect of the testosterone. For the most part, the tetosterone treated rats showed significant hyperkeratosis accompanying the epidermal hyperplasia. However, because of the inherent distortion of the stratum corneum resulting from the processing of paraffin sections, no attempt was made to quantitate the thickness of this layer.

Sebaceous Glands. As part of this study, the effect of the testosterone administration on the sebaceous glands was noted. As to be expected, hyperplasia of the glands occurred. However, it was not until 4 weeks that a majority had sebaceous glands averaging 100% or more larger than the controls. From 8 weeks on, all testosterone rats had sebaceous glands at least double the average sebaceous gland size of the controls.

Alcian Blue Stain. There was no significant increase in Alcian Blue stainable material, presumably revealing acid mucopolysaccharide, in the cutis at any time in the testosterone treated groups as compared to the controls.

DISCUSSION

The principal interest of this study is the effect of testosterone on the epidermis. The stimulatory effect of testosterone on the sebaceous glands has been well documented. Ebling (3) treated female rats with testosterone propionate 1 mg daily subcutaneously for 36 days and produced a marked increase in the size of the sebaceous glands as determined by the average cell count per gland. The number of glands was not increased. An increased cellular activity was noted.

Reports of other investigators on the effect of parenteral testosterone on the epidermis are variable and these discrepancies are explainable due to differences in modes of administration, length of observation periods, and perhaps species differences.

Ebling (2) used 8 mg testosterone cylinder implants, which gave a daily average absorption of 0.229 mg, in castrated male rats. The rats were castrated at 24–25 weeks, implanted with testosterone at 27 weeks, and sacrificed 20–22 days later. Hence the testosterone dosage was only about 0.23 mg daily for 3 weeks. Ebling states that testosterone nearly doubled the rate of epidermal cellular proliferation, as indicated by the mitotic rate, in the castrates but the thickness of the epidermis was not affected. As previously noted, in our data (Table I) a consistent increase in the average epidermal thickness was not noted until 12 weeks. Our dosage of 2.5 mg 3×/week was also significantly greater than Ebling's.

Eartly *et al.* (4) found that testosterone increases the thickness of the epidermis and the number of mitoses in castrated-thyroidectomized male rats. The stratum corneum was markedly increased. The rats were injected subcutaneously with 0.5 mg testosterone daily for 6–7 weeks. It was their interpretation that the increased epidermal thickness was due mainly to an increased cell production and also possibly to an increased life span of the cell.

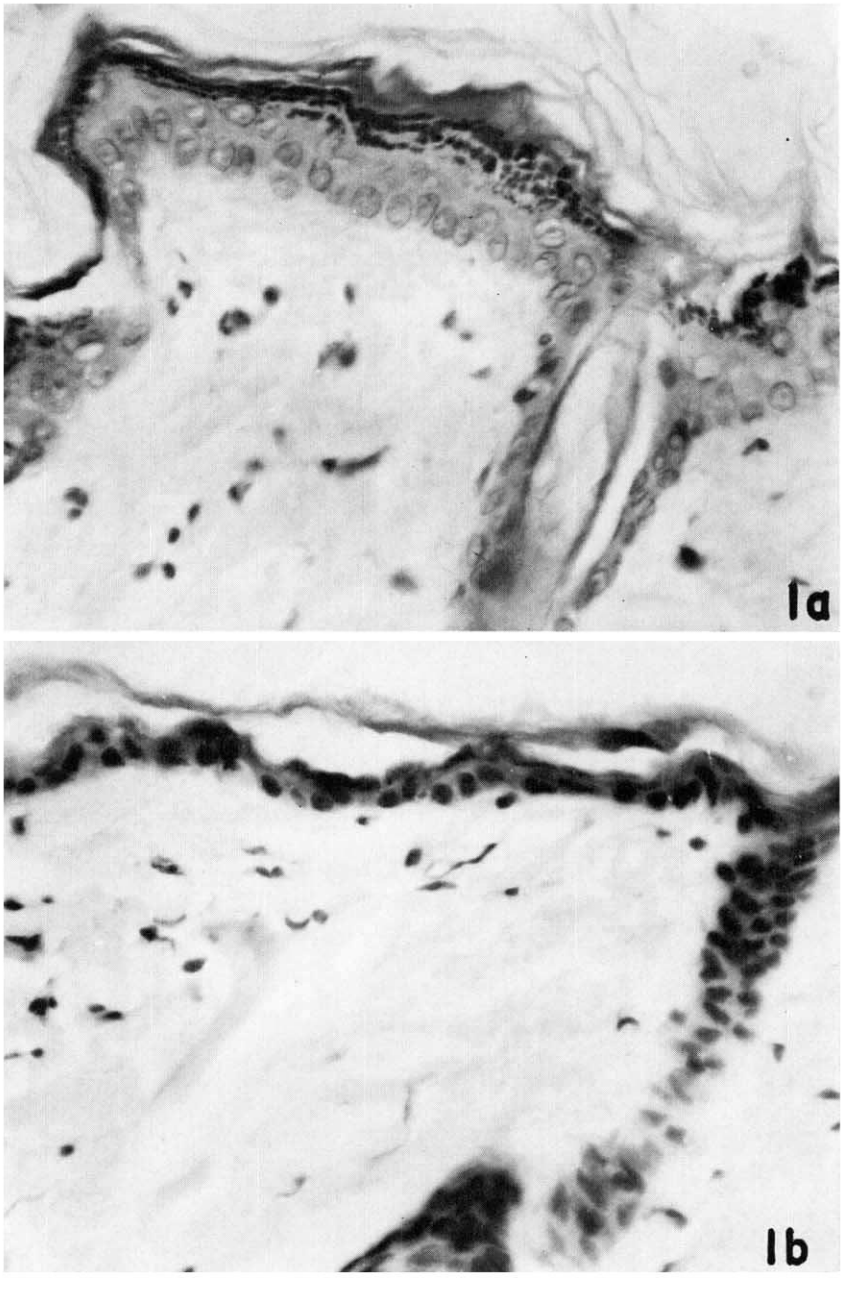

FIG. 1a. Epidermis of rat treated with testosterone 2.5 mg, 3×/week, for 4 weeks. H. & E. × 200.
FIG. 1b. Epidermis of control rat treated with water injections for 4 weeks. H. & E. × 200

Darcis (5) treated female rats with testosterone propionate 1 mg daily for 10 days, IM. This produced a significant increase in the mitotic rate of the mucosa of the vagina and rectum, and the epidermis. Epidermal thickness was not measured.

Topical application of androgens has been reported to produce epidermal hyperplasia.

Montagna *et al.* (6) produced an increase in mitotic activity, epidermal thickening and hyperkeratinization in rabbits' ears by the topical application of strong solutions of testosterone propionate. Papa and Kligman (7) treated the axillae of aged men and women with 1% testosterone propionate cream for one year. A thickening of the epidermis and a sharper definition of the basement membrane was noted.

This study failed to reveal a significant increase in acid mucopolysaccharides in the testosterone treated rats as revealed by the Alcian Blue stain. Allalouf and Bei (8) applied a topical oil solution of testosterone propionate to rat skin. After 7 days of therapy there was a significant increase in uronic acid and a slight increase in hexosamine as determined chemically. However, after 14 days of therapy there was only a slight increase in uronic acid and not of hexosamine.

It may be noteworthy that the stimulatory effect of testosterone on the epidermis was maintained for 25 weeks, the length of the experiment. Testosterone is apparently capable of producing long term effects on epithelial tissues in addition to its well known action on sebaceous glands and sex organs.

SUMMARY

Administration of aqueous testosterone suspension, 2.5 mg, 3×/week, intramuscularly to young intact female rats for 25 weeks, resulted in accentuation of the granular cell layer and increased thickness of the epidermis. However, this epidermal thickening was consistently significant only after 12 weeks of treatment.

REFERENCES

1. Jennings, W. A.: X-Rays: half-value thickness range 0.01–8.0 mm of Al. Brit. J. Radiol., Suppl. No. 10, pg. 1, 1961.
2. Ebling, F. J.: The action of testosterone on the sebaceous glands and epidermis in castrated and hypophysectomized male rats. J. Endocrin., *15:* 297, 1967.
3. Ebling, F. J.: Sebaceous glands. I. The effect of sex hormones on the sebaceous glands of the female albino rat. J. Endocrin., *5:* 297, 1948.
4. Eartly, H., Grad, B. and LeBlond, C. P.: The antagonistic relationship between testosterone and thyroxine in maintaining the epidermis of the male rat. Endocrin., *49:* 677, 1951.
5. Darcis, L.: Influence de la testosterone sur l'activite mitotique du vagin, du rectum et de la peau chez la ratte. Annales d'Endocrin., *23:* 545, 1962.
6. Montagna, W., Kenyon, P. and Hamilton, J. B.: Mitotic activity in the epidermis of the rabbit stimulated with local applications of testosterone propionate. J. Exp. Zool., *110:* 379, 1949.
7. Papa, C. M. and Kligman, A. M.: The effect of topical steroids on the aged human axilla. p. 177, Advances in Biology of Skin, Vol. 6. Ed., Montagna, W., Pergamon Press, Oxford, 1965.
8. Allalouf, D. A. and Bei, A.: Effect of percutaneous application of testosterone propionate and of estradiol benzoate on the total acid mucopolysaccharides of rat skin. Endocrin., *69:* 210, 1961.